An acute toxicity test of
Bio-synthesized Organic Germanium

1. Mouse - An acute oral toxicity test

2. Rat - An acute oral toxicity test

September 15, 1994

Experimental Animal Laboratory,
Research Institute of Animal Medicine College of Veterinary Medicine,
Chungbuk National University, Korea

Report

This report submit as a research paper about acute oral toxicity test to mouse and rat of the GERANTI (Bio-synthesized Organic Germanium) that was manufactured by Geranti Pharm Ltd.

September 15, 1994

Chief researcher: A college of veterinary medicine, Chungbuk University.
A dean professor Kim chang ki
Research institution: The research institute of animal medicine, Chungbuk University
A head assistant professor Ji Cha Ho
Responsible person of research: The research institution of animal Medicine,
A college of veterinary medicine, Chungbuk University.
An assistant professor Kang Jong Ku

1. Self Description of The veterinary medicine research institute

Title	Anti-cancer and Immuno-enhancement Effects of the GERANTI (Bio-synthesized Organic Germanium)			
Client	Client: Geranti Pharm Ltd. president Shon Tsang Uk			
	Address : 678-20 Daiji B/D Yuksamdong kangnamgu Seoul			
Research institution	The name of research institution : A college of veterinary medicine, Chungbuk University			
	Seat : san 48 Gaesindong chongju Chungbuk			
	The name of cheif researcher : A dean professor Kim Chang Ki			
Purpose	This research institute was, depend on the school regulations 344 number, (1993.11.16) relating to the whole of life science, pharmacology, veterinary medicine and medical science. Established for mankind's welfare and health that has been the research on the safety and effectiveness of medicines, agricultural medicines, food and chemicals etc. and the development of a new disease model, an infectious disease of man and animal, as a watchman to defense the health of man and animals.			
Research item	This research institute, relating to the National of Sanitation and Safety Research Institute 65050-188 number (Feb. 24. 1994) has been studied on the efficacy of a medicine, pharmacology test, in addition to the general-toxicity test. genetic-toxicity test, a generative-toxicity test, a local-toxicity test, antigenic test etc.			
Responsible person	Position : The animal medicine research institute, a college of veterinary medicine, Chungbuk University			
	The responsible person of the animal management an assistant professor Kang Jong-Gu			
	Address: san 48 Gaesindong Chongju Chungbuk			
Charge of Animal Control	The responsible person of the animal management an assistant professor Kang Jong-Gu			
	Address: san 48 Gaesindong Chongju Chungbuk			
Member of Researchers	Kang Sin Suk : master course, 2nd term, a college of veterinary medicine department, Chungbuk University			
	Lee Jong Sung: "			
	Oh Myung Ho : "			
	Lee Won Hyung: "			
	Jung Jae Hwang: master course, 1st term, a college of veterinary medicine Chungbuk University			
Research assistant	Yu Young Kyung : 3rd year student, a college of veterinary medicine, Chungbuk University			
	Pak Hak Su : "			
	Kim Mi Young : 2nd year student, a college of veterinary medicine, Chungbuk University			
	Pak Sun Hee : "			
The main facilities	No. of Researcher	10		
	Ground	600 ㎡		
	Building	Building	veterinary college(1)	Breeding room of animals(1)
		Area(㎡)	400	178.2
		The date of construction	Aug. 1990.	Apr. 1994.

2. The main equipment and basic facilities

1. Paraffin temperature maintain apparatus
2. Automatic tissue handling apparatus
3. Embedding center
4. Auto clave
5. Slide heater
6. Microtome for tissue of a living body
7. Paraffin oven
8. Spectrophotometer
9. Multi - using microscope
10. Difference phase Microscope
11. Chimograph of recording type
12. pH meter
13. Clean bench
14. CO_2 incubator
15. Water - bath for constant - te...perature
16. Air- conditioner
17. Computer-80286, 80386
18. Liquid Nitrogen tank
19. Auto dry chemistry analyzer
20. Exp. cage for the toxicity of inspiration
21. Metabolic cage
22. Balance for animals
23. Table of dissection for animals
24. Self -registering thermometer and hygrometer
25. Colony counter
26. Coulter counter for bloods
27. Breeding apparatus with automatic maintainer of temperature and humidity
28. Breeding system of mouse and rat
29. Air cleaner

3. The career of researcher

Name: Kang Jong Ku
Birthday : Dec 14 , 1995
The place of : Chungbuk chongjusi Gaesindong san 48
Work: The animal medicine research institute, a college of veterinary medicine, Chungbuk University Tel : 0431-261-2607, Fax : 0431-267-3150

Research Career

Nov. 1985-May.1987: Toxicity-Pathology part of Japan Highfox safety research Institute (researcher), Take charge of the safety test and new medicine screening.

Apr. 1988-May.1990: An associated researcher of Japan radiation medicine research institute studies on movement of immuno system by radiant and environment substance.(studies on Division and increase of macrophage)

May.1990-Aug.1990: The new medicine screening part of Japan Highfox new drug research institute, New drug development and toxicity test using diseases model (acute. sub-acute chronic. generation, deformity, mutagenic. antigenic, a responsible researcher)

Sep.1990-Sep.1992: The department of veterinary medicine, full-time instructor, Chungbuk University

Oct. 1992-present: A College of veterinary medicine, an assistant professor Chungbuk University

Acting

Sep.1990-present: The councilor of the Korean Experimentation animal society.
Sep.1990-present: The member of the Korean toxicology society.
Sep.1990-present: The member of the Korean toxicity-pathology society
Sep.1990-present: The member of the Korean veterinary medicine society.
Sep.1990-present: The councilor of Japan Highfox society for new medicines
Sep.1990-present: The member of Japan society for toxicology and toxicity-pathology.
Mar.1992-present: National Research Institute of Sanitation and safety, pathology part, an advisory professor and a co-operation research professor.

4. Published Papers (Recent 5 years)

1 Kang J-K , Suzuki,s Nakayama,H . and Goto, N 1989. The multiplication and pathgenicity of plaque mutants of mouse hepatitis virus, MHV, in cultured mouse hepatocytes. Jap. Soc. Vet. Sci.107:89

2 Kang, J-K Azuma, K. , Nakayama, H , and Goto,N 1989 The multiplication of mouse hepatitis virus(MHV-2) mutants in cultured peritoneal macrophage and macrophage-cell line. Jap. Soc Vet. Sci. 108:93

3. Nakayama, H., Morozumi, M., Kang. J-K., and Goto, N. 1990 Hepatic nodular fibrosis in a dog J Vet. Med. Sci 52(4):829

4. Kang Jong-Koo. 1990. Studies on the pathogenicity of mouse hepatitis virus MHV-2, and its mutant strains in vivo and in vitro.(Ph. D. Thesis, University of Tokyo)

5. Goto, N., Kawamoto., Kang. J-K , Uchida, K and Kai, K. 1991. Hepatitogenicity of three plaque purified mutants of hepatotropic mouse hepatitis virus, MHV-2. J Vet. Med. Sci 53(4). 655

6 Kang, J-K 1991 immune responses of peritoneal cavity in mice infected with fulminant mouse hepatitis virus(MHV-2). Korean J. of Lab. Ani. Sci. 7(2):77

7 Kang, J-K , 1991. Mouse hepatitis virus(MHV-2) as a model of the liver infected disease in man. Korean J Vet Sci 7(1):14

8. Kang. J-K., 1991. Peritoneal barrier to the spread of mouse hepatitis virus type-2(MHV-2) in mice. Korean J Vet Sci. 7(2):2

9. Kang. J-K., 1991. Hepatitis and brain lesion in mice infected with mutants of mouse hepatitis virus type-2(MHV-2). Jour Agr. Sci , Chungbuk Nat'l Univ.9(2):11

10. Kim D-J, Han B-S, Cho S-M, Ahn B-W, Moon A, Lee B-Y, Kim C-H Choi K-S, Kang J-K, Lee J-S 1992. The hepatocarcinogenic potential of tamoxifen on the hepatocarcinogenesis induced by dimethylnitrosamine in F344 rats. The report of national institute of safety research 5:261

11 Kang J-K , and Kim C-K, 1992 The effects of BCG pretreatment in pet dogs inoculated experimentally with Mycobacterium bovis. Korean J Vet Sci 332(1):117

12. Kim D-J. Han B-S, Ahn B-W, Chio K-S, Kang J-K, Lim C-H, 1993. Changes in subppoluation of bronchoalveolar lavage Fluid in the pulmonary fibrosis induced by bleomycin or peplomycin. Korean J. Toxicology 9(2):241

13. Kim J-H, Park C-W, Beak Y-G. Lee J-H, Moon B-W, Kim D-J, kang J-K 1993 Experimental model development for hepatotoxicity and carcinogenesis test induced

by MHV/or chemicals. The Report of National Institute of Safety Research 6 41

14. Kim D-J, Kang J-K, Lee J-S. 1994. Modifying effects of verapamil on the pulmonary lesion induced by bleomycin in rats. Korean J Toxicol 10(1) 13
15. Kim D-J, Kim C-K, Kang J-K, 1994. pathological and immunohistochemical changes caused by spontaneous mouse hepatitis virus infection of ICR mice in a Korean breeding colony. Korean J. Lab. Ani Sci 10(1) 95
16. Kang, J-K , and Goto, N 1994 Pathogenicity and multiplication of three mutants of mouse hepatitis virus, MHV-2, in the primary hepatocyte and Kupffer cell cultures J Vet Med Sci (admitted for publication)
17. Kang, J-K , Goto, N. 1994 Response of the cells in the peritoneal cavity and hematopoeitic system to acute infection with mouse hepatitis virus, MHV-2 and its mutants. Hepatology (admitted for publication)

1. An acute toxicity test of Geranti-Germanium in Mouse

Materials and Method

1 Testing Materials

1) Name: Geranti-Germanium

2) Molecular structure: unknown

3) Chemical name: unknown

4) Appearance. a yellow powder

5) pH 6 ~ 8

6) The condition of storage: under 4 degree C

2 The experimental animal

In this test, we were selected ICR mice, because ICR mice have been used widely in safety test for acute, subacute and chronic toxicity test. And there are many the physiology autopsy and toxicological data on ICR mouse. We were used only healthful mice without the decrease of the weight among the laboratory animal that made quarantine and acclimation in laboratory for a week. The discrimination of individual was append a label that is stated the name of responsible person, the period of test, dosage, the number of mice, test number in cage for breeding

The mice were breed a five head each the cage of breeding in polycarbonate cage (175W× 240L × 145Hmm) during the term of testing and acclimation

3 The dosage and the test group

We were set up the highest dosage (5000mg per Kg) that can be administered Geranti-germanium into mice. And Geranti-germanium was diluted in the regular rate (0.5 ratio) with distilled water standardizing this dosage and set up the group of five. The composition of the test group, dosage and liquid measure were the following

The material of administration	Test group	Sex	The number of animal	The animal No.	Dosage (mg/kg)	Administration volume (ml/Kg B.W)
Dilute Geranti with D. W.	GA1	M	5	GAM 1 - GAM 5	5,000	20
		F	5	GAF 1 - GAF 5		
	GA2	M	5	GAM 6 - GAM 10	2,500	20
		F	5	GAF 6 - GAF 10		
	GA3	M	5	GAM 11 - GAM 15	1,250	20
		F	5	GAF 11 - GAF 15		
	GA4	M	5	GAM16 - GAM 20	625	20
		F	5	GAF 16 - GAF 20		
	GA5	M	5	GAM21 - GAM 25	312.5	20
		F	5	GAF 21 - GAF 25		
Control group (D. W.)	GA6	M	5	GAM 26 - GAM 30	0	20
		F	5	GAF 26 - GAF 30		

*G: Intragastrically, A: Acute, M: Male, F: Female

4 The administration of test material

1) The preparation of test solution

We were prepared the test solution that was diluted in distilled water every dosage just before administration and the germanium content measured by phenylfluorone method

2) The route and the method of administration

We were selected oral administration on the basis of the acute toxicity test method In mice case, administrate in the stomach using the Sonde The calculation of dosage was fixed on the basis of mice weight. And the powder of Geranti was dissolved in distilled water It was only distilled water in control group And the maximum dosage was set up 5000㎎ per ㎏ administrate to mice

5 The observation

The day of administration was observed the general symptoms every hour to twelve hours including the change of general state, the symptoms of poisoning, motility appearance, and whether animal was died or not everyday one times from next day to fourteenth day The body weight was measured three times, just before autopsy (after 14 days) after seventh days and just before the administration on all mice. The LD_{50} was calculated by the method of Litechfield-Wilcoxon using computer program pharmacological calculation system

Result

(1) LD_{50} value

When this test material was administrated oral on male and female mice, the example of death was not observed in the maximum dose of 5000㎎/㎏ (0.2㎖/10g B.W.). So the calculation of LD_{50} value was impossible.

(2) The death rate (Table 1)

In case of oral administration, the example of death was not observed during the total period of test in the both sexes.

(3) Clinical symptoms (Table 2)

The clinical symptom was not observed at all tests group for testing period.

(4) The change of body weight (Table 3)

The significant difference of each group was not observed at all tests group for testing period.

(5) The view of autopsy (Table 4)

It was not observed at all the pathological view due to this material in the autopsy on all survival animals.

(6) The histopathological changes

The significant histopathological change was not observed at all due to this material in the autopsy on all survival mice.

Table 1. Mortality of male and female ICR mice administered intragastrically with Geranti-Germanium.

Sex	Dose	Hours after treatmen							Days after treatment													Final
	(㎎/㎏)	1	2	3	4	5	6	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mortality
Male	5,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2,500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1,250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	312.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	0(Control)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
Female	5,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2,500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1,250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	312.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	0(Control)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

Table 2 Clinical Findings of Male and Female ICR Mice administered intragastrically with Geranti-Germanium.

M Male, F .Female

N · No of animals examined

- No abnormality detected

Sex	Dose (mg/kg)	Findings	Hours after treatment							Days after treatment												
			1	2	3	4	5	6	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M	5,000	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2,500	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1,250	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	625	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	312.5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0 (Control)	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
F	5,000	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2,500	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1,250	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	625	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	312.5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0 (Control)	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 3. Body Weights of male and Female ICR Mice administered intragastrically with Geranti-germanium (Mean ± SD · g)

Sex	Days after Treatment	Dose(mg/kg)					
		5,000	2,500	1,250	625	312 5	control
Male	0	25.68 ± 0 36 (5)	24.96 ± 0 40 (5)	25.34 ± 0 52 (5)	25.08 ± 0 45 (5)	25.48 ± 0 73 (5)	25 56 ± 0 59 (5)
	7	33 38 ± 2.56 (5)	30.58 ± 4 10 (5)	33.90 ± 1.21 (5)	33.02 ± 1.66 (5)	30.80 ± 3.55 (5)	29 36 ± 0 47 (5)
	14	35 52 ± 3.57 (5)	31 54 ± 4.65 (5)	35 14 ± 1 38 (5)	32.16 ± 5.03 (5)	35.14 ± 2 50 (5)	33 34 ± 1 14 (5)
Female	0	21.14 ± 0 60 (5)	21 08 ± 0.78 (5)	21 32 ± 0.71 (5)	21.20 ± 0.70 (5)	21.02 ± 0.54 (5)	21 42 ± 0.61 (5)
	7	26.04 ± ˜ 78 (5)	27 22 ± 0.53 (5)	26.82 ± 1.26 (5)	25.60 ± 0.90 (5)	26.80 ± 0.88 (5)	26.18 ± 0 ((5)
	14	27.68 ± 0.99 (5)	29 12 ± 1.27 (5)	28.06 ± 0.80 (5)	28.46 ± 0.47 (5)	25 96 ± 3 13 (5)	27 86 ± 1.02 (5)

Table 4. Gross finding of male ICR mice administered intragastrically with Geranti-germanium

	Dose(mg/kg)					
	5,000	2.500	1.250	625	312 5	control
Brain						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-L						
No of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Heart						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Lung						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Spleen						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Liver						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Pituitary gland						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
other organs						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5

Table 5. Gross findings of female ICR mice administered intragastrically with Geranti-germanium

	Dose(mg/kg)					
	5,000	2,500	1,250	625	312.5	control
Brain						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Heart						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Lung						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Spleen						
No. of observations	5	5	5	5	5	5
No gro _ findings	5	5	5	5	5	5
Liver						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Pituitary gland						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
other organs						
No of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5

Discussion

Geranti-germanium was natural products with *Saccharomyes cerevisiae* containing organic germanium that is biosynthesized in yeast cell. This organic germanium is chemically connected inorganic germanium ions with organic compound such as amino acid or organic acid Also it is contain a very small amount as a trace element in the plant, animal and living body From this century, organic germanium have been known the therapeutic effect on incurable disease including cancer, hypertension, diabetes, heart disease, regressive disease, arthritis and show immune enhancement and detoxification of heavy metals So Recently, The research for incurable disease, especially in cancer and heart disease, using organic germanium is actively progressing in America, Japan and other countnes. In the ways to get organic germanium for human being, one is extracting from the natural product like Ginseng, Formes japonicus etc. And the other is chemical synthesis through reaction of organic acid and germanium dioxide by catalyst. But the former is not cost effective while the other is not completely safe as foods or medicines. Meanwhile, Yeast, as a widely used for brewing, breaking, is not only much affected in diet for several thousand years but also is using widely as a source of supply with rich nutritious containing proteins, vitamins, minerals oneself. Hence, Geranti Pharm Ltd. as a result of research for organic germanium was developed the method of mass production of safe organic germanium using yeast

In th[illegible] study, in order to examine the acute oral toxicity of Geranti [illegible]ermanium in mice since the maximum dosage was 5,000mg/kg. The maximum dosages that can be administrate in both sexes of mice using Sonde into the stomach one times and observed for 14 days. The example of death was not observed in all tests group and impossible the calculation of LD_{50} value. Also it was not observed the change of body weight and clinical symptoms in case the autopsy too. From the whole result, Geranti-germanium was considered as safe material to mice that it was free from harmful. And it was not observed the pathological view since there are no significant clinical symptoms and the example of death.

References

1" The standard of toxicity test for pharmaceuticals"(March 14, 1994, an established by law) that is the regulation (94-3) of the National Sanitation and Safety Research Institute

2 Int J. Radiat Biol. Relat. Stud. Phy. Chem. Med 42(6): 653-659(1982)

3. Anticancer Res., 5(5): 479-483(1985)

2. An acute oral toxicity test of Geranti-Germanium in rat

Materials and Method

1. Test Material

1) Name: Geranti-Germanium

2) Molecular structure. unknown

3) Chemical name: unknown

4) Appearance: a yellow powder

5) pH : 6~8

6) The condition of storage: under 4 degree C

2. The experimental animal

In this test, we were selected SD rat, because SD rat have been widely used in toxicity test including acute, subacute and chronic toxicity test. And there are many the physiology autopsy and toxicological data on SD rat. We were used only healthful rats without the decrease of the weight among the laboratory animal that made quarantine and acclimation in laboratory for a week. The discrimination of individual was append a label that is stated the name of responsible person, the period of test, dosage, the number of rat, test number in cage for breeding.

The rat were breed a five head each the cage of breeding in polycarbonate cage (175W× 240L × 145Hmm) during the term of testing and acclimation.

3. The dosage and the test group

The maximum dosage was set up as 5000㎎ per ㎏ and dosage volume was 0.2ml/10g body weight. And Geranti-germanium was diluted in the regular rate (0.5 ratio) with distilled water standardizing this dosage and set up the group of five. The composition of the test group, dosage and liquid measure were the following

The material of administration	The test group	sex	The No. of animal	The animal No.	Dosage (㎎/㎏)	liquid measure (ml/Kg B.W)
Dilute JY with D. W.	GA1	M	5	GAM 1 - GAM 5	5,000	20
		F	5	GAF 1 - GAF 5		
	GA2	M	5	GAM 6 - GAM 10	2,500	20
		F	5	GAF 6 - GAF 10		
	GA3	M	5	GAM 11 - GAM 15	1,250	20
		F	5	GAF 11 - GAF 15		
	GA4	M	5	GAM16 - GAM 20	625	20
		F	5	GAF 16 - GAF 20		
	GA5	M	5	GAM21 - GAM 25	312.5	20
		F	5	GAF 21 - GAF 25		
Control group (D. W.)	GA6	M	5	GAM 26 - GAM 30	0	20
		F	5	GAF 26 - GAF 30		

G : Intragastrically, A : Acute, M : Male, F : Female

4 The administration of test material

1) The preparation of test solution

We were prepared the test solution that was diluted in distilled water just before administration and the germanium content measured by phenylfluorone method.

2) The route and the method of administration

We were selected oral administration on the basis of the acute toxicity test method. In rat case, administrate in the stomach using the Sonde. The calculation of dosage was fixed on the basis of body weight. And the powder of Geranti was dissolved in distilled water It was administered only distilled water in control group. And the maximum dosage was set up 5000 mg/kg and administrate to rat.

5. The observation

The day of administration was observed the general symptoms from the first day to fourteenth day including the change of general state, the symptoms of poisoning, motility, appearance, and whether animal was died or not. The body weight was measured as mean value from three times in just before autopsy (after 14 days), after seventh days and just before the administration on all rats. The LD_{50} was calculated by the method of Litechfield-Wilcoxon using computer program pharmacological calculation system version4.1.

Result

(1) LD_{50} value

When this test material was administrated oral to male and female rat. The example of death was not observed in the maximum dose of 5000㎎ per ㎏ (0.2㎖/10g B.W.). So the calculation of LD_{50} value was impossible.

(2) The death rate (Table 1)

In case of oral administration, the example of death was not observed during the test penod in both sexes.

(3) Clinical symptoms (Table 2)

The unique clinical symptom due to test material was not observed during the test period in both sexes.

(4) The change of body weight (Table 3)

The significant change of body weight in each group was not observed during the test period in both sexes.

(5) The view of autopsy (Table 4)

It was not observed the pathological abnormality due to this material in the autopsy of survival rat.

(6) The histopathological view

The significant histopathological sign was not observed in the autopsy of survival rat.

Table 1. Mortality of male and female SD Rat administered intragastrically with Geranti-germanium

Sex	Dose	Hours post-treatment							Days after treatment													Final
	(㎎/㎏)	1	2	3	4	5	6	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mortality
Male	5,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2,500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1,250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	312.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	0(Control)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
Female	5,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2,500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1,250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	312.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	0(Control)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

Table 2. Clinical Findings of Male and Female SD rat administered intragastrically with Geranti-germanium

Sex	Dose (mg/kg)	Findings	Hours after treatment							Days after treatment												
			1	2	3	4	5	6	12	1	2	3	4	5	6	7	8	9	10	11	12	13	14
M	5,000	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2,500	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1,250	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	625	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	312.5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	(Control)	-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
F	5,000	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2,500	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1,250	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	625	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	312.5	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
		-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	(Control)	-	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

M : Male, F :Female

N : No. of animals examined

- : No. Abnormality detected

Table 3. Body Weights of male and female SD rat administered intragastrically with Geranti-germanium (Mean ± SD : g)

Sex	Days after treatment	Dose(㎎/㎏)					
		5,000	2,500	1,250	625	312.5	control
Male	0	25.68 ± 0.36 (5)	24.96 ± 0.40 (5)	25.34 ± 0.52 (5)	25.08 ± 0.45 (5)	25.48 ± 0.73 (5)	25.56 ± 0.59 (5)
	7	33.38 ± 2.56 (5)	30.58 ± 4.10 (5)	33.90 ± 1.21 (5)	33.02 ± 1.66 (5)	30.80 ± 3.55 (5)	29 36 ± 0.47 (5)
	14	35.52 ± 3.57 (5)	31.54 ± 4.65 (5)	35.14 ± 1.38 (5)	32.16 ± 5.03 (5)	35.14 ± 2.50 (5)	33.34 ± 1.14 (5)
Female	0	21.14 ± 0.60 (5)	21.08 ± 0.78 (5)	21.32 ± 0.71 (5)	21.20 ± 0.70 (5)	21.02 ± 0.54 (5)	21.42 ± 0.61 (5)
	7	26.04 ± 0.78 (5)	27.22 ± 0.53 (5)	26.82 ± 1.26 (5)	25.60 ± 0.90 (5)	26.80 ± 0.88 (5)	26.18 ± 0.65 (5)
	14	27.68 ± 0.99 (5)	29.12 ± 1.27 (5)	28.06 ± 0.80 (5)	28.46 ± 0.47 (5)	25.96 ± 3.13 (5)	27.86 ± 1.02 (5)

Table 4. Gross finding of male SD rat administered intragastrically with Geranti-germanium.

	Dose(㎎/㎏)					
	5,000	2,500	1,250	625	312.5	Control
Brain						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Heart						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Lung						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Spleen						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Liver						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Pituitary gland						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Other organs						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5

Table 5. Gross findings of female SD Rat administered intragastrically with Geranti-germanium

	Dose(㎎/㎏)					
	5,000	2,500	1,250	625	312.5	Control
Brain						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Kidney-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Heart						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Lung						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Spleen						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Liver						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Adrenal gland-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Pituitary gland						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-L						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Testis-R						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5
Other organs						
No. of observations	5	5	5	5	5	5
No gross findings	5	5	5	5	5	5

Discussion

Geranti-germanium was natural products with *Saccharomyes cerevisiae* containing organic germanium that is biosynthesized in yeast cell. This organic germanium is chemically connected inorganic germanium ions with organic compound such as amino acid or organic acid Also it is contain a very small amount as a trace-element in the plant, animal and living body From this century, organic germanium have been known the therapeutic effect on incurable disease including cancer, hypertension, diabetes, heart disease, regressive disease, arthritis and show immune enhancement and detoxification of heavy metals. So Recently, The research for incurable disease, especially in cancer and heart disease, using organic germanium is actively progressing in America, Japan and other countries In the ways to get organic germanium for human being, one is extracting from the natural product like Ginseng, Formes japonicus etc And the other is chemical synthesis through reaction of organic acid and germanium dioxide by catalyst. But the former is not cost effective while the other is not completely safe as foods or medicines. Meanwhile, Yeast, as a widely used for brewing, breaking, is not only much affected in diet for several thousand years but also is using widely as a source of supply with rich nutritious containing proteins, vitamins, minerals oneself. Hence, Geranti Pharm Ltd. as a result of research for organic germanium was developed the method of mass production of safe organic germanium using yeast.

In this study, in order to examine the acute oral toxicity of Geranti-germanium in rat since the maximum dosage was 5,000mg/kg. The maximum dosages that can be administrate in both sexes of rat using Sonde into the stomach one times and observed for 14 days. The example of death was not observed in all tests group and impossible the calculation of LD_{50} value. Also it was not observed the change of body weight and clinical symptoms in case the autopsy too. From the whole result, Geranti-germanium was considered as safe material to rat that it was free from harmful. And it was not observed the pathological view since there are no significant clinical symptoms and the example of death.

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